Abstract

Separation and purification of compounds (proteins, enzymes, antigen-antibodies, co enzymes etc) from biological sources are very important and may account for up to two thirds of total costs The recent upsurge of interest in biotechnology has aroused renewed consider ation of the range of products of commercial value that might be produced from biological systems Isolation of compounds from biological sources allows products to be produced that are too complex or too costly to be manufactured by chemical synthesis The purification of biological molecules by affinity chromatography is based on the principle of exclusive bi ological recognition The technique offers phenomenal purification factors in a single step and is most suited for the separation of enzymes and cofactors in complex mixtures It has great potential for purifying valuable substances such as gene products and biologically active compounds The process essentially consists of adsorption, washing and elution steps The process could be applied for the separation and purification of expensive antibodies like IgG using immobilized antigens like Protein A

The experimental system chosen for the present study is Concanavalin A (Con A) Sephadex Con A is a plant protein obtained from jackbeans It is purified by affinity chromatography Purified Con A of the present study and Con A obtained commercially are characterized for purity and activity Purity is checked by SDS PAGE and activity by haemagglutination test The values obtained by haemagglutination test are in good agreement with those reported in literature Con A exists as a dimer below pH 5 6 and as a tetramer between pH 5 6 and 7 0 The affinity separation is carried out on the solid adsorbent Sephadex Sephadex is the trade name of dextran crosslinked with epichlorohydrin This essentially consists of glucose and α-1,6 glycosidic linkages Con A will interact with the

glucose as it reacts with polysaccharides Batch adsorption washing and elution experiments are conducted at pHs 3 4 and 6 3 The adsorption profiles are quantitatively discussed in terms of protein recovery efficiency and adsorbent utilization efficiency

Batch equilibrium and kinetic studies were conducted to arrive at the isotherm constants and kinetic rate constants Experimental studies of Con A purification in batch and fixed bed modes were carried out to investigate the effect of the following parameters a) protein concentration b) pH c) particle size d) adsorbent volume e) flow rate The equilibrium and rate constants obtained by the batch studies are used to evaluate the breakthrough curves obtained from the packed bed Adsorption, washing and elution steps are studied The results are compared with those generated by the numerical simulations They are in good agreement with each other The washing and elution steps required less time than that of adsorption step

Mathematical models for the adsorption, washing and elution stages of the following reactor configurations are developed 1) batch 2) fixed bed and 3) membrane In general, film mass transfer, intraparticle diffusion, equilibrium and kinetic interaction, convection and axial dispersion are included in the mathematical models The comprehensive models proposed require numerical computation One point orthogonal collocation method and higher order orthogonal collocation methods are implemented for the numerical simulation experiments One point orthogonal collocation method is an elegant, simple and straightforward numerical technique for arriving at an approximate solution of a model under relevant initial and boundary conditions The tools required for the implementation of the numerical technique are detailed Simulation results are compared with those of analytical solutions and experimental results The validity of the numerical schemes are ascertained The technique is found to be efficient with respect to solution behaviour and computation time

The FORTRAN numerical codes developed for the models in this study are quite efficient both in terms of solution behaviour and computation time One point orthogonal collocation (OPOC) was used to assess the system behaviour Once reasonable success was

obtained, higher order orthogonal collocation (OC) method was implemented

Design strategies are arrived at through protein recovery efficiency and adsorbent utilization efficiency for the batch reactor, protein recovery efficiency, adsorbent utilization efficiency, mass transfer zone and length of unused bed for packed bed reactor, protein recovery efficiency, adsorbent utilization efficiency, mass transfer zone and length of unused membrane for membrane reactor

Experimental and theoretical analysis of the relative performances of a given quantity of affinity adsorbent used in a batch, fixed bed and membrane systems are studied